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The Apparent Turnover of 1-Aminocyclopropane-1-Carboxylate Synthase in Tomato Cells Is Regulated by Protein Phosphorylation and Dephosphorylation

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In suspension-cultured cells of tomato (*Lycopersicon esculentum* Mill.), the activity of 1-aminocyclopropane-1-carboxylate synthase (ACC-S) rapidly increases in response to fungal elicitors. The effect of inhibitors of protein kinases and protein phosphatases on the regulation of ACC-S was studied. K-252a, an inhibitor of protein kinases, prevented induction of the enzyme by elicitors and promoted its apparent turnover in elicitor-stimulated cells, causing a 50% loss of activity within 4 to 8 min in both the presence and absence of cycloheximide. Calyculin A, an inhibitor of protein phosphatases, caused a rapid increase of ACC-S in the absence of elicitors and an immediate acceleration of the rate of ACC-S increase in elicitor-stimulated cells. In the presence of cycloheximide there was no such increase, indicating that the effect depended on protein synthesis. Cordycepin, an inhibitor of mRNA synthesis, did not prevent the elicitor-induced increase in ACC-S activity but strongly reduced the K-252a-induced decay and the calyculin A-induced increase of its activity. In vitro, ACC-S activity was not affected by K-252a and calyculin A or by treatments with protein phosphatases. These results suggest that protein phosphorylation/dephosphorylation is involved in the regulation of ACC-S, not by regulating the catalytic activity itself but by controlling the rate of turnover of the enzyme.

ACC-S catalyzes the first step in the pathway of ethylene biosynthesis in higher plants, the conversion of *S*-adenosylmethionine to ACC. The enzyme usually has a very low level in tissues that do not produce ethylene but is rapidly induced when ethylene is synthesized, and therefore, it is considered the principal regulatory step in the synthesis of the hormone (Yang and Hoffman, 1984; Kende, 1989, 1993).

ACC-S is well known to be induced by de novo protein synthesis, as shown by ^2H_2O density labeling in wounded tomato (*Lycopersicon esculentum* Mill.) fruits (Acaster and Kende, 1983) and in elicitor-treated parsley cells (Chappell et al., 1984). In most cases studied induction appears to be based on increased levels of ACC-S mRNA, as summarized by Kende (1993). However, in suspension-cultured cells of parsley (Chappell et al., 1984) and of tomato (Felix et al., 1991a; Spanu et al., 1993) ACC-S induction was insensitive to inhibitors of mRNA formation, suggesting a posttranscriptional mechanism of regulation.

Rapid turnover appears to be another feature contributing to the regulation of ACC-S, as shown by the rapid decay of enzyme activity after treatment of tissues with cycloheximide. The half-life of ACC-S determined in this way varied from 20 min in tomato leaves (Spanu et al., 1990) and 25 min in mung bean hypocotyls (Yoshii and Imaseki, 1982) to 40 min and 2 h in green and pink tomato fruits, respectively (Kende and Boller, 1981); in tomato cell-suspension cultures the half-life was found to be approximately 40 min (Spanu et al., 1990). ACC-S is inactivated in a time-dependent manner in vitro in the presence of its substrate, *S*-adenosylmethionine (Boller, 1985; Satoh and Ehashi, 1986), and it has been demonstrated that inactivation is due to covalent attachment of the vinylglycine moiety from *S*-adenosyl-L-Met to the active site (Satoh and Yang, 1988, 1989a, 1989b). Mechanism-based inactivation appears to proceed more rapidly for dimeric than for monomeric forms of ACC-S (Satoh et al., 1993). The significance of this mechanism-based inactivation in vivo is a matter of debate: In wounded tomato leaves and in elicitor-treated tomato cells, inactivation in vivo does not appear to be based on this mechanism, since it is not inhibited by aminoethoxyvinylglycine, a competitive inhibitor of the enzyme as well as of the substrate-based inactivation in vitro (Spanu et al., 1990). In contrast, Kim and Yang (1992) provided immunological evidence suggesting that in tomato fruits turnover in vivo can be prevented by competitive inhibitors, and they concluded that substrate-based inactivation occurs also in vivo.

We previously found that the elicitor-dependent induction of ACC-S and of ethylene biosynthesis can be blocked by inhibitors of protein kinases, such as K-252a and staurosporine (Grosskopf et al., 1990; Felix et al., 1991b), and that CA, a potent inhibitor of protein phosphatases 1 and 2A (Ishihara et al., 1989), can mimic elicitors with respect to induction of ACC-S and other responses (Felix et al., 1994). This indicates that protein phosphorylation plays a central role in the regulation of ethylene biosynthesis and ACC-S. Here we report that inhibition of protein kinases with K-252a promotes inactivation of ACC-S, leading to decay rates of 1 order of magnitude greater than previously measured and that inhibition of protein phosphatases by CA prevents inactivation. These findings, which strongly suggest that protein phosphorylation regulates ACC-S inactivation in vivo, are discussed in relation to the biochemical events that underlie regulation of ACC-S and, ultimately, ethylene biosynthesis.

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Abbreviations: ACC-S, ACC synthase; CA, calyculin A.

MATERIALS AND METHODS

Tomato Cell Culture

Tomato (*Lycopersicon esculentum* Mill.) cell-suspension cultures, line Msk8 (Koornneef et al., 1987), were grown on a Murashige-Skoog-type medium as described by Felix et al. (1991a). Cells were used for experiments 7 to 10 d after subculture.

Elicitors, Inhibitors

To induce ACC-S, the cells were treated with 5 μg/mL of an elicitor obtained from yeast extract as described previously (Felix et al., 1991a). Inhibitors were added from stock solutions as follows: Cycloheximide (Boehringer) was dissolved in water at a concentration of 100 mM. Cordycepin (3′-deoxyadenosine, Fluka) was dissolved in warm water (40°C) at a concentration of 80 mM and stored at −20°C. K-252a (Kamiya Biomedical Co., Thousand Oaks, CA) was dissolved in dimethyl formamide at a concentration of 2 mM and stored at −20°C; the stock solution was diluted to 0.5 mM in water immediately prior to treatment. CA (LC Services Corp., Woburn, MA) was dissolved in DMSO at a concentration of 1 mM and stored at −20°C; the stock solution was diluted to 0.1 mM in water immediately prior to treatment. The amount of solvents present in the cell suspension after treatment (0.025% dimethyl formamide and 0.01% DMSO) had no effect on ACC-S activity (not shown).

Assay of ACC-S Activity

Samples of cells (0.8–1.0 mL) were harvested by filtration. After addition of permeabilization buffer (0.25 volume of 150 mM Hepes-KOH buffer [pH 8.0] containing 5 mM DTT, 5% [w/v] Triton-X-100, and 10 μM pyridoxal phosphate), the suspension was immediately frozen on a slurry of solid CO_2 in ethanol and then kept at −80°C. Cells were thawed and washed three times with permeabilization buffer diluted 5-fold with H_2O before measuring ACC-S in the permeabilized cells as described previously (Spanu et al., 1990). To study the effect of inhibitors and enzymes on ACC-S in cell extracts, permeabilized elicited cells were homogenized, pretreated with the various effectors for 10 min, and assayed according to the procedure of Chappell et al. (1984). Enzyme treatments were performed with purified protein phosphatases PP1 and PPA2 from rabbit muscle (kindly provided by Dr. K. Ballmer, Friedrich Miescher-Institut) and with immobilized alkaline phosphatase from bovine intestine (Mobitec, Göttingen, Germany). After the samples were treated with the immobilized alkaline phosphatase, fresh pyridoxal phosphate was added to the enzyme preparation.

RESULTS

The Protein Kinase Inhibitor K-252a Promotes Inactivation of ACC-S

Untreated tomato cell-suspension cultures had a basal ACC-S activity of about 0.01 pkat/g. The addition of an elicitor derived from yeast extract caused an increase in ACC-S activity (Fig. 1); typically, induction started after a lag phase of 10 to 20 min and continued for 3 to 4 h when the activity reached a maximum and then decreased. The protein kinase inhibitor K-252a prevented the increase in ACC-S if added before the elicitor (Felix et al., 1994). When K-252a (0.5 μM) was added 150 min after the elicitor, when ACC-S activity had almost reached its maximal level, the activity decayed rapidly to the baseline. Generally, addition of K-252a to elicitor-treated cells caused a 50% loss of activity within 4 to 8 min (approximately 4 min in Fig. 1). The structurally related protein kinase inhibitor staurosporine, when applied at the same concentration, caused the same rapid inactivation of ACC-S (data not shown). Lower concentrations of K-252a (0.1–0.3 μM) produced a similar decay of enzyme activity initially, but the ACC-S activity started to recover after 10 to 20 min, indicating a loss of inhibitor activity. ACC-S activity induced by different elicitors, such as xylanase (Felix et al., 1994), was inactivated equally rapidly after treatment with K-252a (data not shown).

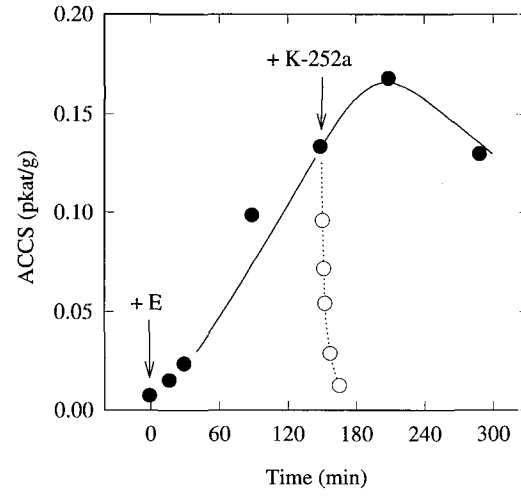

Figure 1. Effect of K-252a on ACC-S activity in elicitor-treated tomato cells. Suspension-cultured cells were treated with elicitor (+E, 5 μg/mL) at t = 0 min; ACC-S was measured for 5 h (●). K-252a (0.5 μM) was added to an aliquot of the cells at t = 150 min, and ACC-S activity was measured for an additional 18 min (○).

In the cells used, cycloheximide has been shown to block induction of ACC-S by elicitors and to cause a decay of its activity with a half-life of 40 to 60 min (Spanu et al., 1990; Felix et al., 1991a). In the experiment shown in Figure 2, cycloheximide (200 μM) was added 60 min after the elicitor and caused a decay of ACC-S activity with a half-life of about 40 min (Fig. 2). When K-252a was added 5 min after cycloheximide, the activity decreased at a rate comparable to that obtained with K-252a alone (Fig. 2). When the protein kinase inhibitor was added 30 min after cycloheximide, the rate of inactivation was reduced approximately 4-fold (data not shown), suggesting that protein synthesis is necessary to maintain the capacity for rapid inactivation of ACC-S.

In our earlier study on the inactivation of elicitor-induced ACC-S (Spanu et al., 1990) we observed that aminoethoxyvinylglycine, a competitive inhibitor of ACC-S that can protect the enzyme from mechanism-based inactivation in vitro,

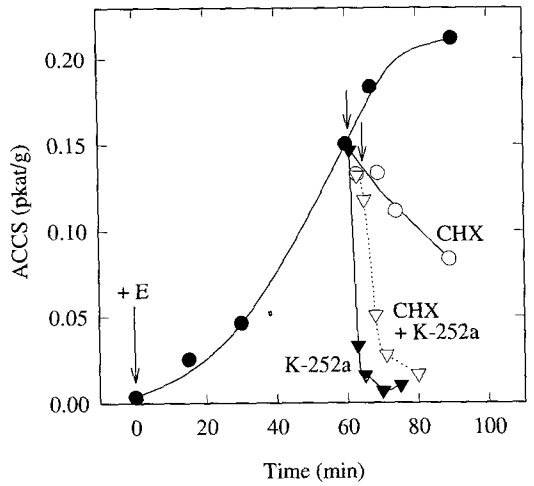

Figure 2. Effect of cycloheximide on K-252a-induced inactivation of ACC-S in elicitor-treated tomato cells. Cells were treated with elicitor (5 μg/mL) at t = 0 min, and their ACC-S activity was monitored (●). Aliquots of the cells were further treated with cycloheximide (CHX, 200 μM, ○) or K-252a (0.5 μM, ▼) at t = 60 min, or first with cycloheximide (t = 60 min) and then 5 min later (t = 65 min) with K-252a (▽).

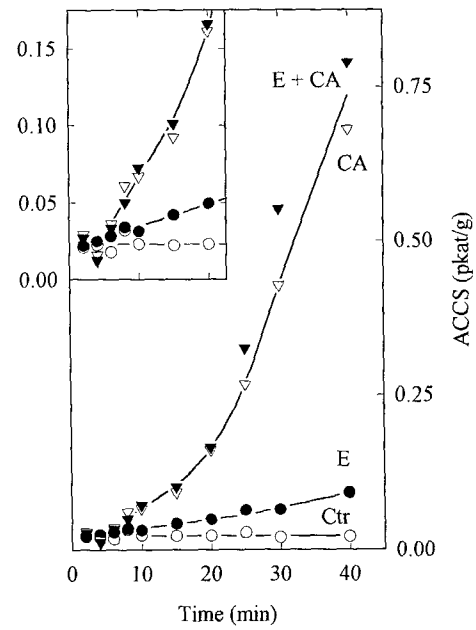

Figure 3. Time course of the increase in ACC-S activity in tomato cells treated with elicitor and/or CA. Cells were left untreated (Ctr, ○) or treated with elicitor (E, 5 μg/mL, ●) or CA (0.1 μM, ▽), or with both elicitor and CA (▼). Inset, Activities measured after short times of treatment shown on an expanded scale.

did not alter the apparent turnover rate in cells treated with cycloheximide. Similarly, there was no difference in the rate of decay of elicitor-induced ACC-S activity caused by K-252a in cells treated in the presence of 50 μM aminoethoxyvinylglycine (data not shown), indicating that the rapid decay of activity was not related to mechanism-based inactivation.

The Protein Phosphatase Inhibitor CA Causes a Rapid Increase in ACC-S Activity

Treatment of the tomato cell suspension with the protein phosphatase inhibitor CA has been found to mimic several aspects of the elicitor response including the rapid increase in ACC-S activity (Felix et al., 1994). We studied the effect of protein phosphatase inhibitors on ACC-S more closely. When cells were treated with CA (0.1 μM), the activity of ACC-S started to increase 8 min after addition of CA, and it increased more steeply than in response to elicitor (Fig. 3). When CA was added together with the elicitor, the increase in ACC-S was approximately the same as with CA alone. When CA was added 120 min after the elicitor (Fig. 4), during the phase of steady increase of ACC-S, the rate of increase rose at least 10-fold almost immediately. As described before (Felix et al., 1994), okadaic acid, another potent inhibitor of protein phosphatase, was much less active in the suspension-cultured tomato cells and was found to induce ACC-S similarly to CA only when applied in concentrations of ≥1 μM (data not shown).

The effect of cycloheximide on the stimulation of ACC-S by CA was investigated. In cells pretreated for 120 min with elicitor the ACC-S activity was about 0.4 pkat/g (Fig. 5). When left without further treatment (control), activity in these cells increased during the 20 min of the experiment to about 0.7 pkat/g. As expected, cycloheximide (added at t = 119 min, Fig. 5) stopped induction, and the enzymatic activity decayed with an apparent half-life of approximately 30 min. Addition of CA (at t = 120 min) resulted in a doubling of ACC-S activity within 3 min (Fig. 5), and ACC-S reached a level of >3 pkat/g within 20 min. Cycloheximide, added 1 min prior to CA, blocked this increase in ACC-S, and activity remained at approximately 0.4 pkat/g.

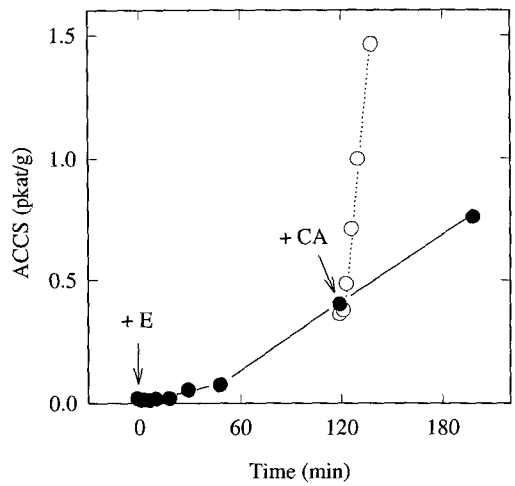

Figure 4. Effect of CA on ACC-S activity in elicitor-treated tomato cells. Cells were treated with elicitor at t = 0 min (+E, 5 μg/mL), and ACC-S was monitored (●). CA (0.1 μM) was added at t = 120 min, and ACC-S activity was measured for 18 min (○).

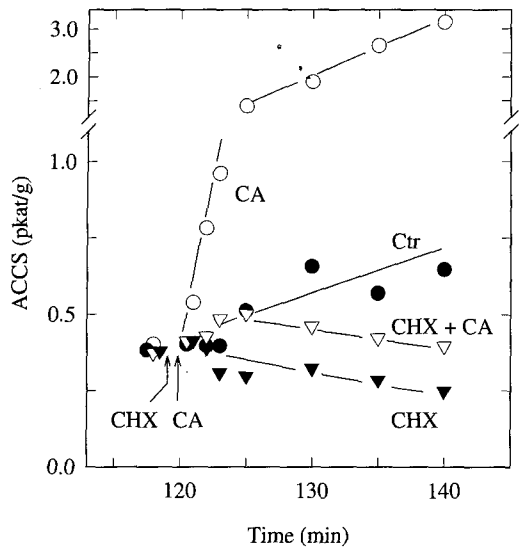

Figure 5. Effect of cycloheximide on the increase in ACC-S caused by CA in elicitor-treated tomato cells. Cells treated with elicitor (5 μg/mL) for 120 min were kept without further treatment (Ctr, ●) or treated with cycloheximide (CHX, 200 μM, ▼) at t = 119 min, with CA (0.1 μM, O) at t = 120 min, or with both in sequence (▽).

Effects of K-252a and CA on ACC-S during Elicitor Induction

The effects of K-252a and of CA were studied at different times after the start of elicitor treatment (Fig. 6). In this experiment, ACC-S activity reached its maximum in the elicitor-treated cells after approximately 3 h (1.5 pkat/g) and then decreased (Fig. 6). When CA was added alone or in combination with elicitor at t = 0 min, ACC-S activity started to increase after a lag of 4 to 8 min (Fig. 3; see also Felix et al., 1994). When CA was added ≥15 min after the elicitor, ACC-S activity started to increase at a higher rate without apparent lag (Fig. 6). The rate of increase, determined from changes observed during the first 8 min after addition of CA, changed over time and was maximal 90 min after elicitor treatment (Fig. 6). K-252 added at t = 0 min with or without elicitor had no effect on the basal level of ACC-S activity (Fig. 6; see also Felix et al., 1994). When K-252a was added after the elicitor, ACC-S activity decreased immediately. The rate of decrease, determined from changes during the first 8 min, strongly increased during the first 90 min and then remained approximately constant (Fig. 6).

K-252a, cycloheximide, and CA had no direct effects on ACC-S activity when added to permeabilized cells or to cell homogenates (data not shown). Treatment of homogenates from elicitor-treated cells with purified protein phosphatases (P1 and PP2A from rabbits) or with an unspecific alkaline phosphatase from bovine intestine did not affect the activity of ACC-S (data not shown).

Effect of Cordycepin on K-252a-Induced Inactivation of ACC-S

Cell-suspension cultures were treated with elicitor either in the presence or in the absence of cordycepin (160 μM, added 15 min prior to the elicitor) (Fig. 7A). In the absence of cordycepin, the elicitor caused an increase in ACC-S activity, which, in this experiment, peaked at approximately 1 pkat/g after 3 h and then decreased. In the presence of cordycepin, the activity of ACC-S increased somewhat more slowly and reached 0.7 pkat/g after 3 h but continued to increase for the duration of the experiment. The pattern shown in Figure 7A was the most typical one among a number of experiments performed in the same way and resembled previous results (Felix et al., 1991a), although in some of the experiments the initial rate of increase in ACC-S was similar or even higher in elicitor-treated cells in the presence of cordycepin than in its absence (data not shown). In all instances, the ACC-S activity kept increasing for a longer time in the presence of cordycepin than in its absence.

When K-252a was added to cell suspensions 60 min after the elicitor (Fig. 7B), the ACC-S activity decreased from approximately 0.3 to 0.05 pkat/g within 8 min. In cells that had been pretreated with cordycepin and elicitor together, K-252a caused a reduction of activity from 0.13 to 0.09 pkat/g within 8 min. Similarly, when CA was added to cells pretreated with the elicitor alone, ACC-S rapidly increased 4-fold from 0.3 to 1.2 pkat/g in 8 min, but when it was added to cells pretreated with cordycepin and elicitor, ACC-S increased only 2-fold, from 0.11 to 0.21 pkat/g in 8 min (Fig. 7C).

DISCUSSION

Regulation of the pathway of ethylene biosynthesis in response to various stress factors has been studied extensively (see Kende, 1989, 1993, for reviews). Usually, whereas the ethylene-forming enzyme ACC oxidase is constitutively expressed at high levels when ethylene synthesis is basal, ACC-

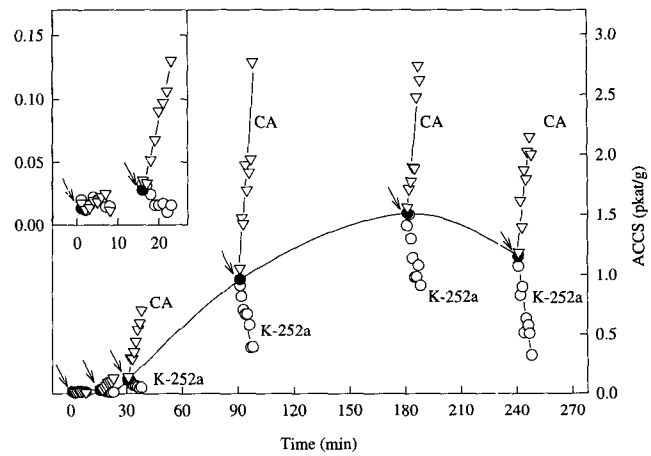

Figure 6. Effect of K-252a and of CA on ACC-S activity in tomato cells treated with elicitor for different times. Cells were treated with elicitor (5 μg/mL) at t = 0 min, and ACC-S activity was monitored at intervals (●). At various times after addition of the elicitor, K-252a (0.5 μM, O) or CA (0.1 μM, ▽) was added. Two aliquots were treated with K-252a or with CA in the absence of elicitor (t = 0 min). The inset shows the early phase of the experiment on an enlarged scale.

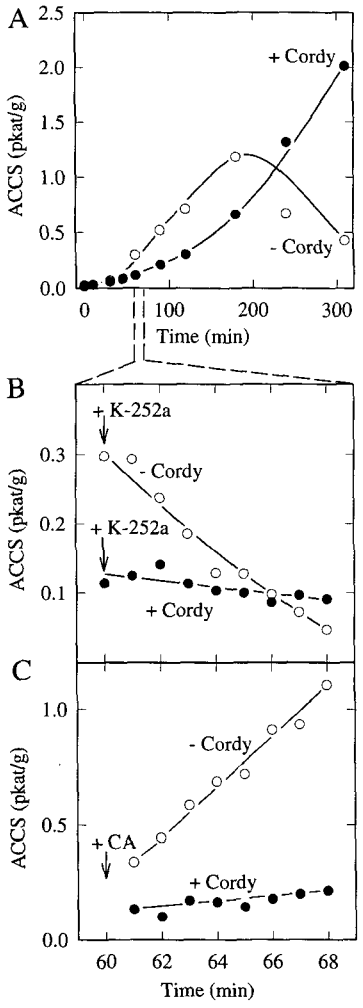

Figure 7. Effect of cordycepin (Cordy) on the increase of ACC-S activity in elicitor-treated tomato cells (A) and on its modulation by K-252a (B) or CA (C). Cells pretreated with cordycepin (160 μM) for 15 min (●) or kept without pretreatment (○) were treated with elicitor (5 μg/mL) at $t = 0$ min. K-252a (0.5 μM) or CA (0.1 μM) were added at $t = 60$ min, and ACC-S was monitored for the next 8 min (B and C).

S is low in these tissues and is markedly induced when ethylene biosynthesis is increased. Therefore, ACC-S is considered the key enzyme for ethylene biosynthesis (Yang and Hoffman, 1984; Kende, 1989, 1993). In the suspension-cultured tomato cells used in the present work, elicitors derived from yeast extract induce ethylene biosynthesis (Felix et al., 1991a), and this is connected to increases in activities of both enzymes of the ethylene biosynthetic pathway, ACC-S (Spanu et al., 1990; Felix et al., 1991a) and ACC oxidase (Felix et al., 1991a; Spanu et al., 1991). Induction of both enzymes is blocked by the inhibitor of protein synthesis, cycloheximide; however, the inhibitor of mRNA formation, cordycepin, blocks only induction of the ACC oxidase but not the induction of ACC-S, suggesting that the former is under transcriptional control and the latter is under posttranscriptional control (Felix et al., 1991a; Spanu et al., 1993). ACC-S activity increases more quickly than ACC oxidase after addition of elicitor, and it also decays more quickly when the elicitor is withdrawn (Felix et al., 1991a), consistent with its key regulatory role in ethylene biosynthesis.

We have examined the potential roles of protein phosphorylation and dephosphorylation in the regulation of ACC-S during elicitation. As shown before, protein phosphorylation is important for transduction of the elicitor signal: staurosporine-type inhibitors of protein kinases such as K-252a (Rüegg and Burgess, 1989) are highly active in the tomato cells and block signal transduction (Grosskopf et al., 1990; Felix et al., 1991b); on the other hand, CA, a potent inhibitor of protein phosphatases 1 and 2a (Ishihara et al., 1989), mimics the elicitor response in vivo (Felix et al., 1994). K-252a also prevents induction of ACC-S by elicitor (Felix et al., 1994), whereas CA alone results in an increase in ACC-S activity in the cells after a lag phase of 4 to 8 min (Fig. 3; Felix et al., 1994). Thus, upregulation of ACC-S is intimately connected to protein phosphorylation events occurring in transduction of the elicitor signal.

The results presented here indicate that changes in the state of protein phosphorylation affect ACC-S activity very rapidly when the enzyme has been induced by elicitor. Addition of K-252a leads to a considerable reduction of activity within minutes, and conversely, CA causes an equally rapid increase of activity. Similar observations have also been made in rice cell-suspension cultures (D.G. Grosskopf, G. Felix, T. Boller, unpublished data), indicating that this effect represents a general phenomenon and is not restricted to tomato cells.

Effects of protein kinase and phosphatase inhibitors on enzyme activities are often due to direct activation/inactivation of the enzymes by reversible phosphorylation (Cohen and Cohen, 1989). According to this model, phospho-ACC-S would be the enzymatically active form, and dephospho-ACC-S would be inactive. However, we discount this simple model based on the following: (a) in the presence of the protein synthesis inhibitor cycloheximide, the CA-induced increase in ACC-S activity is blocked (Fig. 5), suggesting that protein synthesis is needed to sustain the increase in enzyme activity and that there is no pool of inactive (e.g. dephospho-) enzyme; (b) the enzyme is stable both in permeabilized cells and in cell homogenates under conditions in which endogenous protein phosphatases are active; (c) we have not succeeded in affecting ACC-S activity in extracts by treatments with alkaline phosphatase or with protein phosphatases 1 and 2A.

On this basis, we suggest that there is no reversible alteration of the catalytic activity but that changes in protein phosphorylation lead to changes in the turnover of the enyzme. Apparent turnover of ACC-S has been reported previously in experiments in which protein synthesis has been blocked by cycloheximide (Kende and Boller, 1981; Yoshii and Imaseki, 1982; Spanu et al., 1990; Kim and Yang, 1992). We demonstrate here that the apparent turnover in the presence of cycloheximide is accelerated almost 10-fold after addition of K-252a, indicating that decay of ACC-S is strongly enhanced when protein phosphorylation is inhibited. This rapid inactivation proceeds similarly in the presence and absence of aminoethoxyvinylglycine in vivo and, thus,

is not connected to the substrate-mediated inactivation observed in vitro (Boller, 1985; Satoh and Ehashi, 1986; Satoh and Yang, 1988, 1989a, 1989b). The rapid inactivation process is arrested immediately after permeabilization or cell extraction and does not continue in vitro, not even when protein phosphatases are added, indicating that some factor or factors required for the inactivation system are lost upon cell extraction.

As far as the effect of protein phosphorylation is concerned, various models may be proposed (Fig. 8). ACC-S may be the substrate of protein kinases and phosphatases, and its dephosphorylated form, although enzymatically active, may be targeted to a process of inactivation (Fig. 8A). To date it has not been shown whether ACC-S is actually phosphorylated or not. Attempts to immunoprecipitate ACC-S from our cells were unsuccessful. An alternative possibility (Fig. 8B) is that the elements of the inactivation system, the "ACC-S inactivase," are regulated by phosphorylation and dephosphorylation. According to both models, the steady-state of phosphorylation determines the rate at which ACC-S is inactivated. Thus, K-252a, by inhibiting the kinase, would lead to a dephosphorylated "all on" state of ACC-S inactivation, whereas CA would lead to a phosphorylated "all off" state of inactivation.

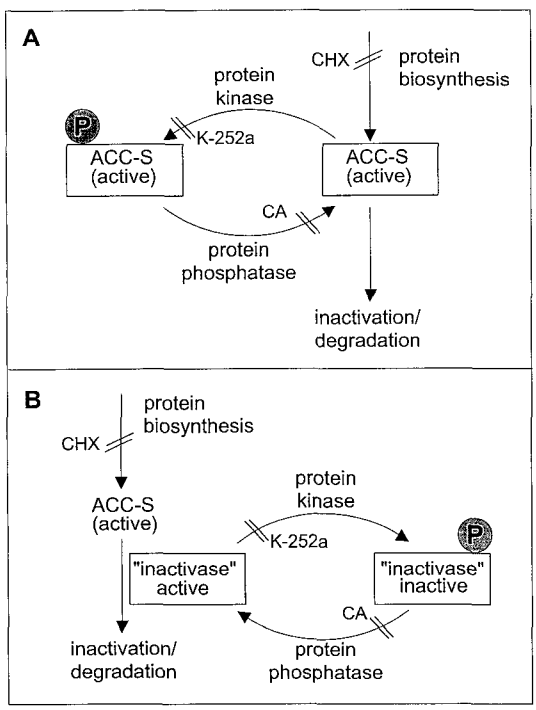

Figure 8. Two alternative models of ACC-S inactivation. A, ACC-S itself is reversibly phosphorylated (P) and dephosphorylated. The state of phosphorylation does not affect enzymatic activity. However, the phosphorylated form (to the left) is protected from inactivation, whereas the dephosphorylated form (to the right) is rapidly and irreversibly inactivated. B, A hypothetical inactivase is regulated by reversible protein phosphorylation. In its dephosphorylated state (to the left), it inactivates ACC-S irreversibly. In its phosphorylated state (to the right), the inactivase is inactive and does not touch ACC-S. CHX, Cycloheximide.

Under the assumption that CA blocks inactivation of ACC-S completely, the rate of ACC-S increase in the presence of CA is an estimate of ACC-S synthesis. When this parameter was measured in the course of an elicitor treatment, it increased strongly for about 90 min and then decreased slowly (Fig. 6), indicating that elicitation leads to an increased synthesis of the enzyme. Conversely, in the presence of K-252a inactivation proceeds presumably at a maximal rate, and the rate of decrease reflects the sum of enzyme synthesis and decay under these conditions. The rapid decrease in ACC-S activity observed after treatment with K-252a demonstrates a very high capacity of the inactivation system in elicited cells. It appears that in elicited cells this system is reduced in its activity by a process involving reversible phosphorylation. Activation and inactivation of this system by inhibitors of protein kinases and protein phosphatases occur within 1 min, indicating a highly dynamic exchange of the phosphate groups involved in its regulation. It remains to be seen to what extent induction of ACC-S activity by elicitors and other stimuli depends on increased synthesis or on reduced decay of the enzyme.

In previous experiments with elicitor-treated cells, it was found that induction of ACC-S is apparently insensitive to inhibitors of mRNA synthesis, e.g. cordycepin, and that levels of ACC-S mRNA exhibit only weak correlations with rates of ACC-S production, indicating that it is regulated in these systems at a posttranscriptional level (Chappell et al., 1984; Felix et al., 1991a; Spanu et al., 1993). The results presented here indicate that this posttranscriptional regulation, or at least part of it, occurs at the level of ACC-S inactivation/degradation. Treatment with cordycepin was found to reduce the potential of CA to stimulate ACC-S increase (Fig. 7C), indicating a reduced ACC-S synthesis. However, it also reduced the rate of decrease in the presence of K-252a (Fig. 7B), indicating that the inactivating system itself requires continuous mRNA and protein synthesis for full activity. As a consequence, the net accumulation of ACC-S in elicited cells remains almost as high in the presence of cordycepin as in its absence at early stages, and it continues to increase for a longer time.

In conclusion, in the cells we have used, protein phosphorylation and dephosphorylation play a decisive role in the tight control of ACC-S activity, primarily through regulation of its inactivation. Although the mechanism of this process remains to be elucidated and is likely to be complex, it provides the cells with an extraordinary potential to rapidly modulate ACC-S activity and, consequently, ethylene production.

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